Characterization of a Novel Hyaluronan-Polyethylene Graft Copolymer for the Delivery of Bioactive Materials

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Statement of Purpose. Bone graft materials (e.g., allograft bones, putties, pastes and bone cements) are employed for a variety of conditions (e.g., arthrodeses and bone defects, resected cysts, maxillofacial malformities). Naturally-derived bone graft materials present concerns due to limited availability and the risk of transmissible disease. Numerous efforts have been directed at creating an alternative supply of graft materials that extend, enhance, and/or replace conventional graft materials. The objective of the work described here was to tailor the use of a novel hyaluronan-polyethylene graft copolymer "HA-*co*-HDPE" to: 1) achieve a bone graft substitute with excellent stability and handling characteristics and 2) to deliver bioactive materials (e.g., demineralized bone matrix (DBM) and chemotherapeutic agents) to both enhance healing and potentially reduce the recurrence of bone tumors. We hypothesize that HA-*co*-HDPE is biocompatible; that it can be formulated to match the rheometric, viscoelastic and clinical handling characteristics of currently available commercial preparations (e.g., DBX® Demineralized Bone Matrix putty, *MTF/Synthes, USA*) and that HA-*co*-HDPE will be amenable to loading with bioactive factors.

Methods. HA-*co*-HDPE was synthesized in two different weight ratios of HA to HDPE: 98:2 (1 M) and 85:15 (10 M) as previously described [1]. Each weight ratio was mixed with phosphate buffered saline to formulate different viscosity solutions resulting in either a putty or a paste. The HA-*co*-HDPE putty was formulated to match DBX® putty; the paste was formulated to a lower viscosity than DBX® putty. All HA-*co*-HDPE mixtures contained 32% (w/w) DBM particles (the generous gift of AlloSource, Centennial, CO). Rheometric properties of the different mixtures were compared; G', G" and tan δ were examined using a dynamic frequency sweep within the samples' linear viscoelastic regions. Viscoelastic properties were investigated using stress relaxation and squeeze flow tests. The relaxation time constant, τ, and steady-state force curve were compared. Specified handling characteristics were evaluated (*ex-vivo* testing performed on ovine femurs) by surgeons who routinely use commercial preparation DBM putties. One-way ANOVAs ($p \leq 0.05$) were run and Student-Newman-Kuels comparisons were performed to test for statistical differences among sample means. Unmodified hyaluronan and polyethylene control materials and test materials were surgically implanted in intramuscular and calvarial sites in 12 adult rats. A 30 day endpoint study was carried out, at which time all materials were excised and histological evaluation and scoring was performed in accordance with ASTM F981-04 by a board certified pathologist.

Results. The G', G" and tan δ (data shown in the table) indicate that the 1 M DBM/HA-*co*-HDPE putty and DBX® putty are virtually identical. The 10 M DBM/HA-*co*-HDPE putty is significantly different from the DBX® putty and the 1 M DBM/HA-*co*-HDPE putty over much of the frequency range for both G' and G", but is not significantly different for tan δ. The 1 M and 10 M DBM/HA-*co*-HDPE pastes are not significantly different from each other.

Material	**0.1 rad/s**	**1.0 rad/s**	**10 rad/s**	**100 rad/s**
Storage Modulus, G' ($x10^5$ dyn/cm^2)				
DBX® Putty	***7.09***	***17.4***	***27.3***	***37.3***
1 M Putty	8.08	17.3	30.3	48.1
10 M Putty	8.70	24.5	48.0	75.8
1 M Paste	3.60	6.04	9.63	14.5
10 M Paste	4.62	8.14	13.2	22.2
Loss Modulus, G" ($x10^5$ dyn/cm^2)				
DBX® Putty	***4.23***	***6.28***	***7.40***	***8.76***
1 M Putty	4.79	8.69	13.4	16.2
10 M Putty	5.32	13.1	21.1	35.1
1 M Paste	1.12	2.00	3.64	6.07
10 M Paste	1.76	3.02	5.82	7.24

The long-time relaxation stresses (t = 400 s) and relaxation time constant, τ, of all samples were not significantly different. For the squeeze flow test, the steady-state slopes were compared and the DBX® putty and 1 M and 10 M DBM/HA-*co*-HDPE putties were not significantly different, nor were the 1 M and 10 M DBM/HA-*co*-HDPE pastes and 10 M DBM/HA-*co*-HDPE putty. The results of the *ex-vivo* handling characteristics test indicated that all formulations of DBM/HA-*co*-HDPE examined were appropriate for use in clinical applications. Histopathological assessment revealed no notable tissue damage and/or negative host response surrounding the implant materials. Implant sites containing HA-*co*-HDPE yielded similar favorable histopathological scores to controls, suggesting that the novel material is biocompatible. Sites containing the antineoplastic agent carboplatin revealed mild to moderate inflammatory response after 30 days.

Conclusions. HA-*co*-HDPE is biocompatible and can be successfully loaded with bioactive materials such as DBM or chemotherapeutics such as carboplatin. The resulting material properties closely approximate those of commercially existing bone graft substitutes.

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References. [1] Cranson, Cody, A Novel Hyaluronan-Polyethylene Graft Copolymer as a Carrier for Demineralized Bone Matrix, M.S. Thesis, Colorado State University, Summer 2008.